

**OFFICE OF NAVAL RESEARCH**

**FINAL REPORT**

**PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS/STUDENTS REPORTS**

for

Grant No. N00014-90-J-1161/P00005

R&T Code 4133030

“Electrochemistry In and At Single Nerve Cells”

Dr. Andrew G. Ewing

The Pennsylvania State University

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June 1, 1996

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OFFICE OF NAVAL RESEARCH  
PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS REPORT

R&T Number: 4133030-09

Contract/Grant Number: N00014-90-J-1161/P00005

Contract/Grant Title: Electrochemistry In and At Single Nerve Cells

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- a. Number of papers submitted to refereed journals, but not yet published: 6
- b. Number of papers published in refereed journals (complete citations included): 4
- c. Number of books or chapters submitted, but not yet published: 1
- d. Number of books or chapters published (complete citations included): 1
- e. Number of technical reports/non refereed papers: 0
- f. Number of patents filed: 0
- g. Number of patents granted: 0
- h. Number of invited presentations (complete citations included): 12
- i. Number of submitted presentations (complete citations included): 3
- j. Honors/Awards/Prizes for contract/grant employees: 0
- k. Total number of Full time equivalent Graduate Students and Post Doctoral associates supported during this period, under this R&T project number
  - Graduate Students: 3
  - Post Doctoral Associates: 0
  - including the number of
    - Female Graduate Students: 2
    - Female Post Doctoral Associates: 0
  - the number of
    - Minority\* Graduate Students: 0
    - Minority\* Post Doctoral Associates: 0
  - and the number of
    - Asian Graduate Students: 1
    - Asian Post Doctoral Associates: 0
- l. Other funding (agencies, grant titles, amount received this year, total amount, period of performance, and a brief statement regarding the relationship of the research to the ONR grant are included)

The letter and an appropriate title is used as a heading for each list, e.g.:

- b. Published Papers in Refereed Journals; or
- d. Books and Chapters published.

\*Minorities include Blacks, Aleuts, AmIndians, Hispanics, etc. NB: Asians are not considered an under represented or minority group in science and engineering.

## Part I

### a. Papers Submitted to Refereed Journals (and not yet published)

1. Multiple Classes of Catecholamine Vesicles Observed During Exocytosis from the *Planorbis* Cell Body, G. Chen, A. G. Ewing, *Brain Res.*, in press, ONR support.
2. Observation and Quantitation of Exocytosis from the Cell Body of a Fully Developed Neuron in *Planorbis corneus*, G. Chen, P. F. Gavin, G. Luo, A. G. Ewing, *J. Neurosci.*, in press, ONR, NSF, and NIH support.
3. The Latency of Exocytosis Varies with the Mechanism of Stimulated Release in PC12 Cells, S. E. Zerby, A. G. Ewing, *J. Neurochem.*, in press, ONR and NSF support.
4. Electrochemical Monitoring of Individual Exocytosis Events from the Varicosities of Differentiated PC12 Cells, S. E. Zerby, A. G. Ewing, submitted to *Brain Research*, ONR and NSF support.
5. Characterization of the Effects of varying the pH and Monomer Concentration on Poly(oxyphenylene) Insulating Films on Carbon Fiber Electrodes, C. E. MacTaylor, A. G. Ewing, submitted to *Electroanalysis*, ONR support.
6. Picoliter Beakers for Capillary Electrophoresis and Voltammetry, P. J. Beyer, S. D. Gilman, R. Lee, A. G. Ewing, submitted to *J. Am. Chem. Soc.*, ONR and NIH support.

### b. Papers Published in Refereed Journals

- 1.\* Voltammetry of Adenosine After Electrochemical Treatment of Carbon Fiber Electrodes, T. K. Chen, T. G. Strein, T. Abe, A. G. Ewing, *Electroanalysis*, 6 (1994) 746-751. ONR and NSF support.
- 2.\* Laser Activation of Microdisk Electrodes Examined by Fast Scan Rate Voltammetry and Digital Simulation, T. G. Strein, A. G. Ewing, *Anal. Chem.*, 66 (1994) 3864-3872. ONR and NSF support.
- 3.\* Amperometric Monitoring of Stimulated Catecholamine Release from Rat Pheochromocytoma (PC12) Cells at Zeptomole Level, T. K. Chen, G. Luo, A. G. Ewing, *Anal. Chem.*, 66 (1994) 3031-3035. ONR and NSF support.
4. Amphetamine Redistributes Dopamine from Synaptic Vesicles to the Cytosol and Promotes Reverse Transport, D. Sulzer, T. K. Chen, Y. Y. Lau, H. H. Kristensen, S. Rayport, A. G. Ewing, *J. Neurosci.*, 15 (1995) 4102-4108, ONR and NIH support.

\* Note: These papers were reported as "not yet published" in last years report.

c. Book Chapters Submitted for Publication

1. Contemporary Problems in Biology: Cell Constituent Analysis, P. J. Beyer, R. Lee, M. R. Wood, N. Winograd, A. G. Ewing, In "Nanofabrication and Biosystems: Integrating Materials Science, Engineering, and Biology," H. Hoch, L. W. Jelinski, H. Craighead, eds., Cambridge Press, NY (1995), in press, ONR, NSF, and NIH support.

d. Books or Chapters Published

1. Voltammetry and Amperometric Probes for Single Cell Analysis, A. G. Ewing, T. K. Chen, In "Neuromethods: In Vivo Monitoring I," R. Adams, ed., Humana Press, Clifton NJ, (1995), pp. 269-304, ONR and NSF support.

e. Technical Reports etc.

None

f. Patents Filed

None

g. Patents Granted

None

h. Invited Presentations at Topical or Scientific/Technical Society Conferences

1. A. G. Ewing, "Analytical Chemistry of Single Nerve Cells," 4th International Workshop on Bioanalysis, Lawrence, KS, July 12, 1994.
2. A. G. Ewing, "Electrochemical Detection for CE of Samples from Picoliter Beakers," American Chemical Society National Meeting, Symposium Honoring Fred Anson for the 1994 Analytical Award in Electrochemistry, Washington, DC, August 22, 1994.
2. A. G. Ewing, G. Chen, "Electrochemical Monitoring of Dopamine Exocytosis from Giant Dopamine Cells of Planorbis Corneus," American Chemical Society National Meeting, Symposium Honoring R. Mark Wightman for the 1994 Analytical Chemistry Award in Chemical Instrumentation, Washington, DC, August 23, 1994.
4. A. G. Ewing, G. Chen, S. Zerby, "Release of Catecholamines from Vesicles in Cell Bodies of Processed Neurons," 6th International Conference on *in vivo* Methods, Seignosse, France, September 17, 1994.

5. A. G. Ewing, "Analytical Chemistry in Picoliter Biological Environments: Voltammetry and Capillary Electrophoresis," 25th Meeting of the Royal Spanish Chemical Society, Vitoria-Gasteiz, Spain, September 26, 1994
  6. A. G. Ewing, G. Chen, "Electrochemical Detection on Quantal Release from Nerve Cell Bodies," 21st Annual FACSS Conference, Symposium on Analytical Methods in Neuroscience, St. Louis, MO, October 4, 1994.
  7. A. G. Ewing, P. J. Beyer, "Electrochemistry in Picoliter Beakers," 21st Annual FACSS Conference, Symposium on Electrochemical Sensor: Solution to 'Real World' Problems, St. Louis, MO, October 4, 1994.  
Group, Rockville, MD, October 27, 1994.
  8. A. G. Ewing, "Monitoring Exocytosis at Single Cells: Is it Quantal? Is it Localized to the Synapse? Can it be Modulated Pharmacologically?," University of Göteborg, Janssen Neuroscience Colloquium, Göteborg, Sweden, February 6, 1995.
  9. A. G. Ewing, P. F. Gavin, S. Zerby, G. Chen, "Dynamic Separations and the Detection of Catecholamines Released from Quantal of Single Nerve Cells," 1995 Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Symposium on Chemical Dynamics at Single Cells, New Orleans, LA March 6, 1995.
  10. A. G. Ewing, G. Chen, S. Zerby, R. Zhou, D. Sulzer, "Electrochemical Monitoring of Exocytosis at Single Cells: Physiology and Pharmacology," Swedish Academy of Pharmaceutical Sciences, Symposium on Electrochemical Detection, Stockholm, Sweden, March 21, 1995.
  11. A. G. Ewing, "Electrochemical and Separations-Based Approaches to Single Cell Analysis," State University of New York at Buffalo, Department of Chemistry, Buffalo, NY, April 21, 1995.
  12. A. G. Ewing, "Electrochemical and Separations Based Approaches to Single Cell Analysis," University of Cincinnati, Chemistry Department Colloquium, Cincinnati, OH, May 12, 1995.
- i. Contributed Presentations at Topical or Scientific/Technical Society Conferences
1. S. E. Zerby, J. L. Zeiders, T-K. Chen, A. G. Ewing, "Electrochemical Monitoring of the Dynamics of Muscarinic Receptor - Mediated Catecholamine Release," 1995 Pittsburgh Conference on Analytical Chemistry, New Orleans, LA, March 7, 1995.
  2. G. Chen, D. Gutman, A. G. Ewing, "In Vivo Electrochemical Determination of Exocytosis from Nerve Cell Bodies," 1995 Pittsburgh Conference on Analytical Chemistry, New Orleans, LA, March 7, 1995.

3. P. J. Beyer, R. Lee, S. D. Gilman, A. G. Ewing, "Picoliter Beakers for Sample Holders in Voltammetry and Capillary Electrophoresis," 1995 Pittsburgh Conference on Analytical Chemistry, New Orleans, LA, March 8, 1995.

j. Honors/Awards/Prozes

None

k. Number of Graduate Students Receiving Full or Partial Support on ONR Contract

Total 3                                      Minorities 0                                      Asian 1

Number of Postdoctoral Associates Receiving Full or Partial Support on ONR Contract

Total 0                                      Minorities 0                                      Asian 0

l. Other Funding

Agency: Camille and Henry Dreyfus Foundation  
Title: Camille and Henry Dreyfus Teacher Scholar Award  
Amount: \$50,000 (\$10,000 for 94-95)  
Date: April 15, 1990 - April 15, 1995  
Support is not related to ONR support

Agency: National Institutes of Health  
Title: Capillary Electrophoresis - Single Cell Neurochemistry  
Amount: \$430,070 (\$143,521 for 95-96)  
Date: July 1, 1995 - June 30, 1998  
Support is for capillary electrophoresis development for single cell analysis

Agency: National Institutes of Health  
Title: Molecular Imaging of Biomaterials  
Amount: \$694,102 Co-PI with N. Winograd (my research group received approx \$40,000 support from this grant in 94-95)  
Date: September 30, 1992 - September 29, 1995  
This grant deals with development of TOF SIMS for imaging frozen cell membranes and vesicles

Agency: National Science Foundation  
Title: Microanalytical Methods in Neurochemistry  
Amount: \$401,000 (\$191,000 for 94-95)  
Date: July 1, 1994 - November 30, 1997

This grant deals with development of electrophoresis in small rectangular channels for dynamic separations

## Part II

### a. Principal Investigator

Andrew G. Ewing

### b. Current Telephone Number

(814) 863-4653

### c. Cognizant ONR Scientific Officer

Dr. Robert Nowak

### d. Brief (100 - 200 words) Description of Project

This project concerns the development, characterization and application of ultrasmall electrochemical probes for microenvironments. Schemes are under investigation to construct electrodes with 50-nm total tip diameter. These electrodes will be developed to carry out electrochemical experiments in the smallest microenvironments, including single biological synapses. In addition, methods are under development to construct artificial synapses by chemical patterning and directed growth onto an electrode. A large part of our current effort is aimed at monitoring catecholamine release from single exocytotic events as a means to examine cellular pharmacology and chemistry. Major goals of this project are A) to develop ultrasmall electrodes for placement in the attoliter volume of a single synapse, B) to develop chemical patterning schemes on small electrodes or electrode arrays to guide axonal growth, C) to develop and characterize methods allowing voltammetry in lithographically fabricated picoliter and femtoliter microvials, D) to develop an electrogenerated chemiluminescence based system for simultaneously imaging individual exocytosis events occurring across a cell surface. These methods will allow insights into both neurotransmitter exocytosis and chemical reactivity of single molecules.

### e. Significant Results (50 - 100 words)

This has been an exciting year in electrode and microvial development. Electrodes with ultrasmall total tip diameter insulated with a phenol-allylphenol copolymer have been characterized providing an optimum pH and monomer concentration for the thinnest films. Optically transparent picoliter microvials have been constructed and used. In addition, several extremely important discoveries have been made related to catecholamine exocytosis events have been monitored from varicosities along the neurites in PC12 cell and several experiments have shown that the time between stimulation and exocytosis is dependent on the type of receptor



stimulated. Another exciting discovery is that multiple types of vesicles release catecholamines from the cell body of the dopamine neuron in *Planorbis corneus*.